October 19, 1948.

Dr. H. B. Newcombe,
Atomic Energy Project,
Chalk River, Ontario.

Dear Howard,

I've just read yourvpaper in Genetics on the phenotypic delay in E. coli B. There can be little dispute with your conclusions, and the only point I could make would be that I would be a little less hesitant about suggesting a basis for the difference between the magnitude of the phenotypic delay in your experiments, and in the radiation material: segregation of the recessive resistance allels, certainly in nuclear separation, and possibly at finer levels also.

If you are still interested in the problem, and have the opportunity to pursue it at your present location, you might like to hear about some material which has newly been developed in coli K-12 which should make possible a <u>direct</u> verification and measurement of the phenotypic delay. It is impossible to put all the details down in a letter, but essentially the story is this:

While working out some details of the inheritance of resistance to T1, I ran across a stock (spontaneous mutant?) which gives prototrophs on crossing, some of which are <u>diploid</u> and <u>heterozygous</u> for various factors. When cultivated on minimal medium, these heterozygotes are propagated as such, as most of the segregants carry nutritional deficiencies from the parents. On complete medium, especially on lactose EMB, the segregation is very striking, the heterozygotes giving rise to mosaic Lac+;Lac- colonies. By cultivation on complete medium the segregants are readily separated, and a study of them shows that segregation is accompanied by crossing over. The capacity to produce heterozygotes is trans-

mitted at least to some of the segregants, so that I have been able to synthesize stocks heterozygous for a number of different factors, including resistance to T1. Other evidence I can't go into in detail points to the existence of a deficiency for several loci (in a segment between Bi and Lac) in one of the chromesomes; since this would result in an inviable segregant, the segregation of factors near the deficient segment is not 1:1, but strongly biassed in favor of the alleles not coupled with it. The deficiency is established by the hemizygosity of the loci opposite it. Heterozygotes may be homozygous for some loci different in the parents, as can be shown by using several fermentative differences: e/g. one is Xyl+/Lac- Xyl-Lac+. That Lac is homo- rather than hemi-zygous in thise cases is shown by obtaining Lac+ reverse mutations in these stocks; they then segregate for Lac- and Lac+.

The important point for your work is that the phage resistance alleles generally are recessive, V_1^r/V_1^s being sensitive to T1, but giving resistant segregants. The degree of segregation in a culture is readily determined by plating it on lactose EMB and observing the proportion of mosaic (i.e. of heterozygous cells) colonies, and a comparison of the time course of the genetic segregation, so marked, and of the development of resistant cells, by the methods you have used, should give a direct measure of phenotypic lags, without the complexities of rather intangible mutation rates. Unfortunately, V_{1a} is probably in the deficient segment, but it may be possible to obtain double heterozygotes $V_1^r\ V_{1a}^s/V_1^s\ V_{1a}^r$, which would be most suitable for such a study. I have some involving V_1 and V_{1c}, but these are not so satisfactory as V_{1c}^r sometimes gives a rather indeterminate reaction with T1.

Let me know if you would be interested to carry out such experiments, and I will send you more details and try to set up the necessary stocks,

With best regards,

Yours sincerely,

Joshua Lederberg
Assistant Professor of Genetics

/s